Starch-encapsulated, soy-based, ultraviolet-absorbing composites with feruloylated monoacyl- and diacylglycerol lipids[☆]

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Abstract

Ferulic acid is a hydroxy cinnamic acid derivative found ubiquitously throughout the plant kingdom, is especially abundant in rice and corn bran, and possesses excellent ultraviolet (UV) and antioxidant properties. Ferulic acid was enzymatically incorporated into soybean oil to form feruloylated monoacyl- and diacylglycerols (FAG). The FAG possess the UV-absorbing and antioxidant properties of ferulic acid but are water insoluble and extremely lipophilic. These characteristics make FAG attractive in the cosmeceutical industry as an all-natural replacement for petroleum-based sunscreen active ingredients and antioxidants. The FAG were synthesized from the transesterification of soybean oil with ethyl ferulate catalyzed by the commercial lipase, Novozym 435 (*Candida antartica* lipase B). The FAG were encapsulated as microdroplets within a starch matrix via steam jet cooking (140 °C and 225 kPa). Up to 50% (w/w) of the feruloylated lipids was encapsulated into the starch matrix with the microdroplets ranging in size from 1 to 10 μm. Transmittance and irradiance measurements of UV radiation (300–400 nm) through thin films of neat FAG (not manipulated after FAG synthesis) and starch-encapsulated FAG showed that the FAG retained its ultraviolet-absorbing efficacy after steam jet cooking. Furthermore, starch-encapsulation of the FAG was found to enhance the ultraviolet absorbance of the feruloylated lipids. When encapsulated at 50% (w/w) in the starch matrix, one-half of the coverage (mg/cm^2) of FAG was required to block the same amount or more UV radiation as neat FAG. The starch-encapsulated FAG was formulated as an aqueous dispersion without the need for emulsifiers or surfactants. The dispersions were drum dried to a powder and shown to be easily reconstituted into water dispersions without the loss of ultraviolet-absorbing efficacy.
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1. Introduction

An all-natural sunscreen active ingredient has been derived from two natural plant components, ferulic acid and soybean oil (SBO) (Compton and Laszlo, 2002). The "green", enzymatic transesterification between the ethyl ester of ferulic acid, ethyl ferulate (EF), and SBO produces a mixture of feruloylated monoacyl- and diacylglycerols (FAG) that are the major constituents of
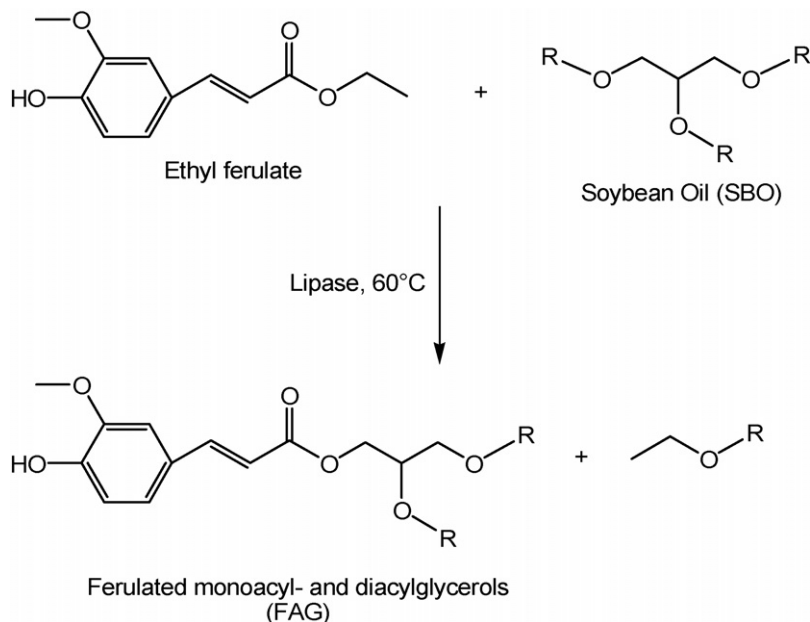
Scheme 1. Transesterification of SBO with ethyl ferulate to form FAG. R = fatty acid or H.

an all natural, soy-based sunscreen (Scheme 1) (Laszlo et al., 2003). Ferulic acid is a phenolic compound, a member of the cinnamic acid family, that is found esterified in most higher plants with hemicelluloses, lignins, and phytosterols. Thus, ferulic acid is a common component of the human diet. The feruloyl moiety of the FAG has a strong ultraviolet A (UVA) and B (UVB) absorbance, whereas the acylglycerol portion of the FAG provides water resistance (Compton and Laszlo, 2002). These characteristics make FAG a suitable all-natural replacement for commercially used, petroleum-based sunscreen active ingredients and antioxidants.

An obvious use of FAG is as a broad UV-absorbing active ingredient in sunscreens, health and beauty aids, and daily-wear cosmetics. Formulation and commercialization of FAG into such products are currently being investigated. However, less obvious but important applications also include using FAG as an adjuvant to provide natural UV protection in paints, coatings, and insecticidal and herbicidal biocontrol agents. As most insecticidal and herbicidal agents are aqueous based, it is necessary to formulate water-based systems containing FAG that allow sprayable, uniform films to be prepared. Initial trials to emulsify FAG into aqueous-based agricultural spray formulations using conventional surfactants have met with limited success. The resultant sprays do not provide uniform coverage needed in agricultural adjuvant applications. An improved, more reliable method

for incorporating FAG into aqueous-based agricultural sprays is needed.

An interesting class of highly stable, water dispersible starch–lipid composites has recently been developed at our facilities by co-jet cooking starch with lipophilic materials such as vegetable oils under excess steam conditions (Knutson et al., 1996; Eskins and Fanta, 1997, 1999; Fanta et al., 1999). The resulting composites can readily contain up to 50% (w/w) lipophilic material relative to the starch component and are water dispersible over a wide range of dilution. Previous publications (Fanta and Eskins, 1995; Eskins et al., 1996; Fanta et al., 1999) describe the preparation, properties and microscopic analysis of these composites and show that the composites consist of lipid droplets having typical diameters in the $1–10\,\mu m$ range coated with a thin film of firmly bound starch at the oil–water interface. The water dispersed starch–oil composites can be drum dried to produce oil microencapsulated in starch flakes that are easily reconstituted in water to again form smooth, stable dispersions. These starch–oil composites provide an ideal method to carry lipophilic components into aqueous systems without the need for additional emulsifiers and stabilizers.

The work herein details the characterization of starch-encapsulated FAG (SE-FAG) composites prepared by excess steam jet cooking. Our goal was to encapsulate the FAG in a starch matrix that would be easily dispersible in aqueous media while maintaining the UV-absorbing

efficacy of the FAG. The irradiance of UV light through SE-FAG films was determined to verify that the FAG remained efficacious after undergoing the jet cooking and drum drying processes.

2. Experimental

2.1. Materials

All materials were used as purchased unless otherwise noted. Novozym 435 was obtained from Novozymes North America (Franklinton, NC). Wesson brand SBO was obtained from a local grocery store. Ethyl ferulate (ethyl 4-hydroxy-3-methoxycinnamate, EF) was purchased from Shanghai OSD (Shanghai, China). Genu pectin DD-slow set Z was obtained from CP Kelco (Atlanta, GA). Waxy cornstarch (Waxy no. 1) was obtained from A.E. Staley Mfg. Co. (Decatur, IL). All other reagents and solvents were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. FAG synthesis

The packed-bed bioreactor synthesis of FAG has been detailed previously (Laszlo et al., 2003). A solution of EF (40 g) dissolved in SBO (160 g) was circulated over a bed of Novozym 435 (34 g) at 60 °C for 144 h. Byproducts and unreacted starting material were removed by molecular distillation at 120 °C.

2.3. SE-SBO and SE-FAG composites

In a typical preparation, a mixture of waxy starch (400 g; moisture content approximately 10%) containing pectin (20 g) was added to deionized water (1500 ml) and stirred in a stainless steel Waring blender (model 37BL84; Dynamics Corporation of America, New Hartford, CT). The resulting slurry was delivered to the jet cooker utilizing a Moyno progressing cavity pump (Robbins Meyers, Springfield, OH) at a flowrate of about 1 l/min. The starch slurry and steam were combined in a Penick and Ford hydroheater (Penford Corp, Cedar Rapids, IA). Cooking temperature was 140 °C using steam supplied at 448 kPa, and the hydroheater backpressure set at 275 kPa. About 2000 ml of the starch solution (solids content: 17–18%, as determined by freeze-drying accurately weighed amounts of the solution in duplicate) was collected. FAG (197 g) was added to the starch solution (1000 g) and the mixture blended at high speed in a Waring blender for approximately 1 min. This starch–oil suspension was then fed through the jet cooker under the conditions just described. Approximately 1900 ml of

white opaque dispersion was collected and allowed to cool in the dark. The dispersion had a solids content of 12–13%. The solids content varied between experiments due to dilution of the cooked dispersion with variable amounts of condensed steam. These aqueous SE-FAG dispersions were drum dried on a pilot-scale double drum dryer (model 20; Drum Dryer and Flaker Company, South Bend, IN) heated with steam at 206.8 kPa (135 °C). The flake-like product was then milled by passing it through a Retsch centrifugal grinder (Brinkman Instruments Inc., Des Plains, IL).

2.4. FAG and SE-FAG films

FAG films were formed by dissolving 5–30 mg of FAG in ethanol (1 ml) and dispensing the solution into 5-cm diameter polished quartz crystal dishes (Quartz Scientific Inc., Fairport Harbor, OH) then allowing the solvent to evaporate in air at room temperature for 24 h, protected from light.

SE-FAG films were formed by dispensing the aqueous SE-FAG dispersions across the top of 4.5 cm × 4.5 cm polished quartz crystal slides (Quartz Scientific Inc., Fairport Harbor, OH) and drawing the dispersion across the slide to form a thin coating using a wet film applicator rod (Paul N. Gardner Company Inc., Pompano Beach, FL) having a 1.27×10^{-2} cm film path thickness. The slides were placed under a box to exclude light while the water was removed by evaporation in air at room temperature for 16 h. The coverage (mg/cm^2) of the FAG and SE-FAG films was determined by the weight difference of the dishes and slides before and after film formation. Desiccation of the films over calcium carbonate did not appreciably alter the weight of the films.

2.5. Scanning electron microscopy

Aqueous SE-FAG dispersions were poured onto glass Petri dishes and allowed to air-dry into thin films. The films were removed from the dishes and fractured to expose the internal structure, then extracted in 100% ethanol for 16 h. Extracted samples were attached to aluminum SEM stubs using conductive tape so the fractured surface could be viewed, sputter-coated with gold–palladium, then examined utilizing a Jeol 6400 V scanning electron microscope operating at an accelerating voltage of 15 kV.

2.6. Transmittance

The transmittance of ultraviolet radiation (300–400 nm) through FAG and SE-FAG films was measured

using a Heraeus Suntest CPS Xenon lamp (Kendro Laboratory Products, Asheville, NC) and a portable LI-1800 Spectroradiometer (LI-COR Biosciences, Lincoln, NE). The transmittance ($T = P/P_0$) was calculated as the ratio of the power of UV radiation transmitted through the films (P, mV) to the power of UV radiation transmitted through air (P_0, mV). The irradiance (E, J/m^2 s) of the transmitted UV radiation through the films was calculated by integration of the area under the transmittance curves. Irradiance values presented are the average of multiple films ($n \geq 3$) of the same coverage. The transmittance and irradiance data were baseline corrected for the quartz crystal plates and slides ($T = 0.99$).

3. Results and discussion

3.1. Preparation of SE-FAG composites

Stable dispersions of the waxy starch-feruloylated monoacyl- and diacylglycerols (SE-FAG) composites were successfully prepared via the excess steam jet cooking process. Although a starch/FAG weight ratio of approximately 1:1 was used in all the experiments reported here, compositions having a starch/FAG weight ratio varying between 1:1 and 10:1 were also effectively prepared. The SE-FAG aqueous dispersions could be drum dried to give a flowable powder that was easily redispersed in water using a Waring blender. Both the resultant SE-FAG composites produced by jet cooking and the redispersed drum dried composites gave smooth, uniform dispersions that were similar in their physical characteristics to starch–soybean oil composites routinely prepared in our laboratories (Fanta et al., 1999). More specifically, we found freshly prepared aqueous SE-FAG dispersions containing 50% (w/w) FAG had a pH ranging between 3.6 and 4.0 and a viscosity of 196 cps. Upon standing for 3 months, the viscosity was found to increase to approximately 300 cps. By light microscopy (data not shown) the aqueous dispersions consisted of FAG microdroplets ranging in size from 1 to 10 μm that did not coalescence over time. These SE-FAG composites are an effective method to carry FAG into aqueous media without the use of surfactants or stabilizers.

To assess the size and distribution of the FAG droplets within the SE-FAG films, the composite films were examined utilizing scanning electron microscopy (SEM). Accordingly, dried SE-FAG films were fractured to expose the internal structure of the film and then extracted to reveal the oil domains as voids in the fracture surface. Fig. 1 shows detailed SEM micrographs of these oil extracted fracture surfaces for an SE-FAG film that
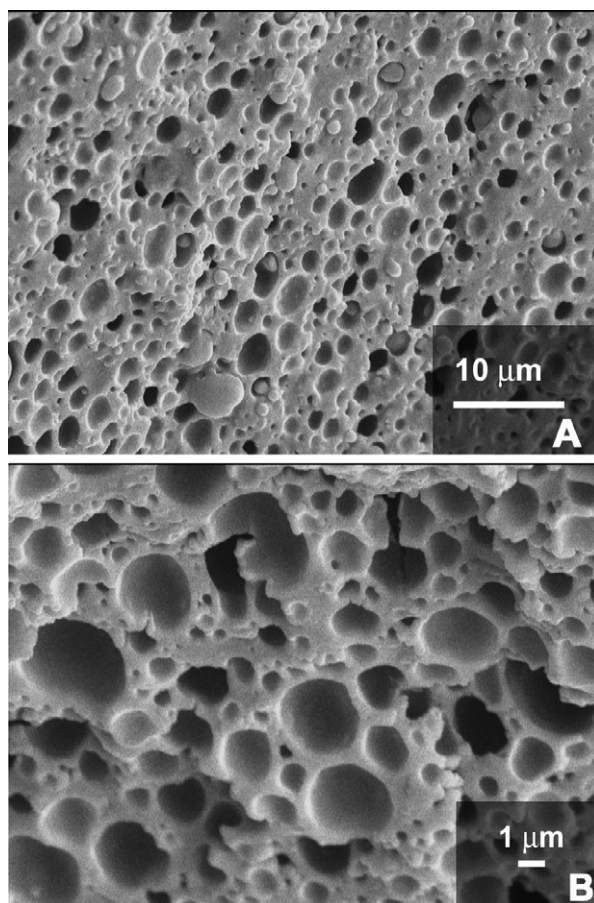

Fig. 1. Scanning electron micrographs of the internal structure of ethanol extracted, air-dried SE-FAG films. Fractured exposed surface for (A) 2000× and (B) 5000× magnification with scale bar = 10 and 1 μm, respectively.

contained approximately 50 parts of FAG per 100 parts of waxy starch. As can be seen, removal of the FAG by the ethanol extraction left voids in the fractured surface where the FAG microdroplet domains resided. Also, it can be seen that the FAG is uniformly dispersed throughout the starch water phase as droplets ranging in size of approximately 0.5–5.0 μm. The shape of the voids is indicative of the spherical shape of the microdroplets and the smooth texture within the voids is consistent with the accretion of starch onto the smooth surface of the FAG droplets.

3.2. UV transmittance through SE-FAG films

It was necessary to determine whether FAG maintained their UV-absorbing efficacy after being subjected to the extreme temperature, pressure and shear conditions produced during the jet cooking process and while
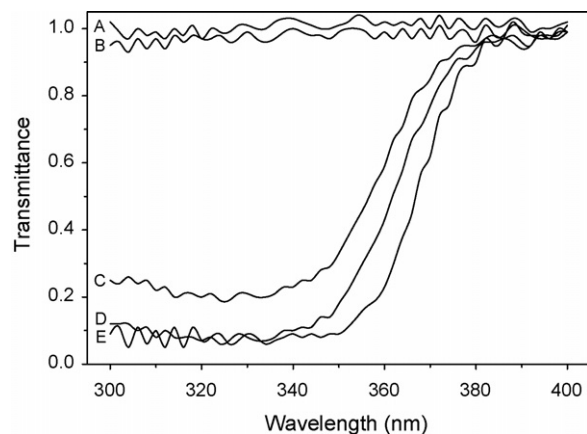
Fig. 2. Transmittance spectra of FAG and SE-FAG films on quartz slides compared with the control films. The spectra were obtained after less than 1 min exposure to a xenon lamp (300–400 nm). The films and their coverage (mg/cm^2) on the quartz slides: (A) SBO 1.38 mg/cm^2; (B) SE-SBO composite (51% (w/w) oil/starch), 1.05 mg/cm^2; (C) FAG, 0.35 mg/cm^2; (D) FAG, 0.65 mg/cm^2; (E) SE-FAG composite (52% (w/w) oil/starch), 0.68 mg/cm^2.

being encapsulated into a starch matrix. Thin films of FAG and SE-FAG were formed on polished quartz crystal slides, and the transmittance of UV radiation from 300 to 400 nm through the films was compared. The FAG films were translucent, whereas the SE-FAG films were slightly opaque. UV radiation was produced by a xenon lamp set to simulate the energy experienced at 12:00 p.m. in central Illinois during summer sun exposure. The transmittance was measured as the ratio of the UV energy transmitted through the films to the UV energy transmitted through air. The transmittance data were baseline corrected for the quartz slides, which had a transmittance of 0.99 over the 300–400 nm region. Lower transmittance corresponds to superior UV absorbance by the films.

The transmittance of UV radiation through FAG and SE-FAG films is shown in Fig. 2. Although the UVB region extends to 290 nm due to the limit of our detector, the transmittance could only be measured from 300 nm and above. Examination of the transmittance spectra of the FAG and SE-FAG films, however, indicates that the absorption of UV radiation from 290 to 300 nm would likely not change appreciably. The data also indicates that the UV absorbance of the SE-FAG films resulted from the FAG in the composite and not the starch, because the starch-encapsulated SBO (SE-SBO) control films permitted almost complete transmittance of the UV radiation. Unexpectedly, we found that encapsulating FAG into the starch matrix enhanced the UV-absorbing ability of the FAG. This enhancement was clearly seen when the UV transmittance between the 0.68 mg/cm^2

SE-FAG film and the 0.35 mg/cm^2 FAG film were compared (Fig. 2, curves C and E). Although the SE-FAG film had a higher UV absorbance, the SE-FAG composite contained about 1:1 oil to starch ratio (w/w), so the amount of FAG coverage provided by the SE-FAG was actually 0.35 mg/cm^2. Thus, the UV absorbtivity of a given quantity of FAG can be increased by formulating it into the starch matrix. The amount of FAG in the control film had to be doubled to 0.65 mg/cm^2 to obtain the same UV absorbance as the SE-FAG film (Fig. 2, curves D and E). These results confirmed that the FAG not only survived the steam jet cooking processes, but the resulting SE-FAG composite films also enhanced the UV absorbtivity relative to neat FAG films.

3.3. UV-absorbing stability of SE-FAG films

Film irradiance values were determined over time to investigate the UV-absorbing stability of the FAG and the SE-FAG films. The irradiance, the energy transmitted to a surface per unit area (J/m^2 s), was obtained by integrating the transmittance spectrum of a film. Fig. 3 compares the initial irradiance values of SE-FAG films to control films and a blank quartz slide. Lower irradiance values correspond to greater UV absorbance. The quartz slide allows the complete transmittance of UV radiation ($T = 0.99$) over the entire 300–400 nm range, and this corresponds to an irradiance of 98.0 J/m^2 s, the highest value possible in our experiments. As the irradiance data shows, SBO and the SE-SBO films have very similar values as the quartz slide (Fig. 3). Thus, the effec-
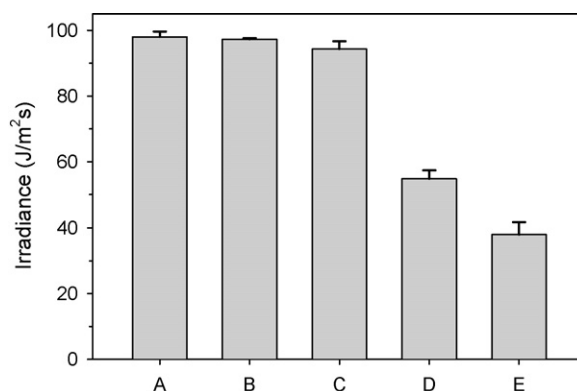

Fig. 3. Irradiance through FAG and SE-FAG films on quartz slides compared with the control films. The irradiance was obtained after less than 1 min. exposure to a xenon lamp (300–400 nm). The values reported are the means of $n \geq 3$ films, and the bars indicate standard deviations. The films and their coverage (mg/cm^2) on the quartz slides: (A) quartz slide; (B) SBO, 1.39 mg/cm^2; (C) SE-SBO composites (51% (w/w) oil/starch), 1.74 mg/cm^2; (D) FAG, 0.23 mg/cm^2; (E) SE-FAG composites (51% (w/w) oil/starch), 0.32 mg/cm^2.
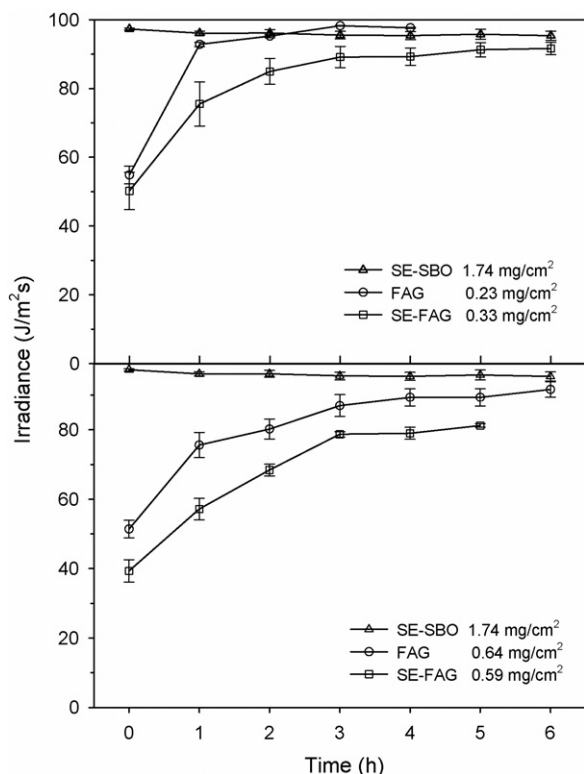
Fig. 4. Irradiance through FAG and SE-FAG films on quartz slides over time. The irradiance was obtained by exposure to a xenon lamp (300–400 nm). The irradiance values reported are the means of $n \geq 3$ films, and the bars indicate standard deviations. The films and their coverage (mg/cm^2) on the quartz slides are—(A) ($\triangle$) SE-SBO composites (51% (w/w) oil/starch), 1.74 mg/cm^2; ($\bigcirc$) FAG, 0.23 mg/cm^2; ($\square$) SE-FAG composites (51% (w/w) oil/starch), 0.33 mg/cm^2; (B) ($\triangle$) SE-FAG composites (51% (w/w) oil/starch), 1.74 mg/cm^2; ($\bigcirc$) FAG, 0.64 mg/cm^2; ($\square$) SE-FAG composites (52% (w/w) oil/starch), 0.59 mg/cm^2.

tive UV absorbance of SBO and the starch matrix was negligible. The unexpected improvement in the SE-FAG film absorbtivity (37.4 J/m^2 s at 0.33 mg/cm^2) relative to the FAG film (54.2 J/m^2 s at 0.23 mg/cm^2) was readily seen by comparison of their irradiances. Because the SE-FAG films used in these experiments contained ca. 50% (w/w) FAG to starch, the actual FAG coverage provided by the SE-FAG films was only 0.16 mg/cm^2. Therefore, the SE-FAG films contained 30% less FAG than the neat FAG control films, but produced a 16.8 J/m^2 s lower irradiance value. These data, again, illustrate the synergistic effect between the starch and FAG in the SE-FAG composites to improve the UV-absorbing efficacy of FAG.

In addition to improving the initial UV absorbtivity of FAG, microencapsulating the FAG into the starch matrix prolonged the FAG efficacy over time. Fig. 4A shows that at a surface coverage of 0.23 mg/cm^2, neat FAG quickly

lost its UV absorbtivity within 1 h of exposure to the UV radiation, rendering it as ineffective as the SE-SBO control film. However, when FAG was applied at a surface coverage of 0.33 mg/cm^2 as a SE-FAG composite containing 48% FAG its UV absorbance was extended for more than 4 h before it degraded to the irradiance of the SE-SBO control. The superior UV stability of the SE-FAG films was even more pronounced at higher coverages (Fig. 4B). It is necessary to stress that the SE-FAG films contained 30 and 50% less FAG than the corresponding neat FAG (Fig. 4A and B, respectively). This indicates that microencapsulation of FAG within the starch matrix resulted in one-half the quantity of FAG providing equal or better UV stability over time than the non-encapsulated FAG.

3.4. Mechanism of enhanced UV absorbance of SE-FAG films

Because encapsulating FAG within a starch matrix enhances its UV absorbance and stability, three plausible explanations as to how the starch coating improves the UV absorbance of the FAG oil droplets can be envisioned: (1) the starch provides a protective barrier for the FAG and lessens photocatalyzed oxidation, (2) the starch acts as a stabilizer by accepting energy from the photo-excited FAG, retarding deleterious photo-degradative reactions, or (3) incident UV irradiation is reflected and refracted as it passes through the films due to distributional and configurational arrangements of the starch coated oil droplets, thereby lengthening the light's path through the film. It is also conceivable that all three mechanisms were in operation simultaneously. Although the first two explanations are plausible, the enhancement of the FAG UV absorbance is most likely attributable to the increased pathlength of the UV photons through the SE-FAG films. A photon experiences refraction at the film's air/FAG interface due to the change in refractive index from air into the FAG film. Once in the film, a photon is directed toward the quartz slide unless it encounters a FAG molecule, in which case it is absorbed. Given equivalent amounts of FAG, the pathlength of the radiation is the only variable that can account for the increase in absorbance observed with the SE-FAG films, as related by Beer's law, $A = \varepsilon bc$, where A is the absorbance, ε the absorption coefficient, b the pathlength, and c is the concentration. When FAG is encapsulated in starch spheres, a photon experiences several refractions at the starch/oil interface with each starch sphere it encounters in the film, which increases the photon's overall pathlength through the film. This increases the probability of a photon encountering a FAG molecule and being absorbed
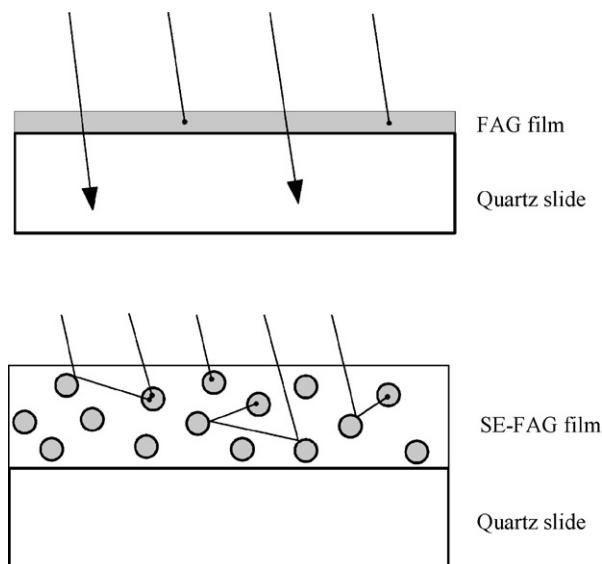
Fig. 5. Depiction of UV radiation path through the FAG and SE-FAG films on quartz slides. The SE-FAG spheres are suspended in a starch matrix. The projected pathlength through the SE-FAG films is longer because of refraction of photons due to the change in refractive indices at the starch–oil boundary.

(see Fig. 5). Thus, a film with an equivalent amount of FAG will have a higher absorbance when the FAG is encapsulated in the starch composite. A similar method to increase the UV absorbance of sunscreen active ingredients was previously reported where hollow spheres made from styrene/acrylate copolymers were used to lengthen the path of UV radiation through the sunscreen films (Jones et al., 1997; Chang et al., 2002). While the starch coating around the FAG oil droplets in the SE-FAG films may have provided some protection against photo-oxidative and photo-degradative decomposition, the increased UV absorbance was most likely due to the increased pathlength of the radiation through the films. However, increased photostability also was observed. The photostability-enhancing mechanism is not known.

4. Conclusions

Feruloylated monoacyl- and diacylglycerols (FAG) were jet-cooked under excess steam conditions with starch and water to form composites consisting of FAG microdroplets encapsulated within starch shells dispersed in water. The FAG in starch aqueous dispersions contained up to 50% of the FAG relative to the starch and did not phase separate into the respective oil, starch and water components even on prolonged standing. Films formed by the evaporative deposition of the starch–oil dispersions onto quartz slides had superior UV absorbance and stability than the equivalent quantities of neat FAG formed into films. The enhanced UV absorbance was speculated to be the result of the increased pathlength experienced by the radiation through the starch–oil films.

These starch–oil composites provide an ideal way for carrying the UV-absorbing oil into aqueous systems without the need for additional emulsifiers and stabilizers. The FAG–starch composites form even, uniform films. These characteristics make the FAG–starch dispersions an ideal mechanism for formulating the UV-absorbing oils into aqueous-based agricultural sprays where they could be used as a natural UV protectant for UV sensitive insecticidal and herbicidal biocontrol agents.

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